

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

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In re Patent Application of:  
Shunichi Shiozawa et al.

Application No.: 10/501,259

Confirmation No.: 7532

Filed: July 9, 2004

Art Unit: 1634

For: DISEASE SUSCEPTIBILITY GENE FOR  
RHEUMATOID ARTHRITIS, PROTEIN  
THEREOF, EVALUATION METHOD AND  
EVALUATION KIT FOR EVALUATING  
ONSET POSSIBILITY OF RHEUMATOID  
ARTHRITIS BY USING THOSE, AND  
REMEDY AND CURING MEDICINE FOR  
RHEUMATOID ARTHRITIS

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Examiner: S. C. Pohnert

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Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

**DECLARATION UNDER 37 CFR 1.132**

The undersigned, Shunichi Shiozawa, declares as follows:

1. I am a resident of Japan. I am an inventor or co-inventor of one or more of the claims of the above-referenced patent application ("the Application"). I have a Ph.D. degree from Kobe University Graduate School of Medicine and an M.D. degree from Kobe University Medical School.

2. I am familiar with the Application and with pending claim 4 thereof. I am also familiar with the Office Action issued from the U.S. Patent Office on April 27, 2007.

3. I am also familiar with certain experiments which were carried out in my

laboratory.

4. In one experiment, PCR was performed as described below to obtain a partial sequence of Angiopoietin-1 from whole RNA of whole blood of RA patients and healthy subjects.

In this experiment, whole RNA was extracted from 300 µl of whole blood of each RA patient and healthy subject with the use of an RNA extraction kit provided by GENTRA system Inc.

In accordance with standard methods, reverse transcription was carried out using Oligo dT primer so as to synthesize 1st strand cDNA. The 1st strand cDNA was used as a template, and a region including a mutation site therein was amplified with the use of the below-described primers in accordance with a 2-step RT-PCR method. 1 µl of a product obtained in the first step was used as a template in the second step. A PCR product thus obtained was subjected to sequence analysis and chain length analysis using standard methods, in order to judge presence or absence of insertion/deletion of the three bases No. 805 to No. 807 in the Angiopoietin-1 gene.

First step

Sense primer: 5'-CCACCAACAACAGTGTCTT-3'

Anti-sense primer: 5'-CAGCTTGATATACATCTGCACAG-3'

Second step

Sense primer: 5'-CAACCTTGTCAATCTTTGC -3'

Anti-sense primer: 5'-CAGCTTGATATACATCTGCACAG-3'

5. The results of the experiment performed in Paragraph 4 are shown in the

Table below:

|                    | Sporadic RA  | Control    |
|--------------------|--------------|------------|
| 3bp deletion Homo  | 11 (9.6%)    | 4 (4.7%)   |
| Hetero             | 71 (61.7%)   | 66 (77.6%) |
| 3bp insertion Homo | 33 (28.7%*1) | 15 (17.6%) |
| N                  | 115          | 85         |

\*1  $p = 0.07$  (There is a significant trend.)

All of them are  $\chi^2$  test results.

As described in the Table, the result of the RT-PCR show that, as compared with the healthy subjects, there is statistically a significant trend in the proportion of RA patients with mutation in homozygotes of 3-base-insertion in No. 805 to No. 807 in Ang-1.


6. In my view, it is possible to evaluate the onset or onset possibility of rheumatoid arthritis in a human subject by detecting the homozygous presence or absence of a gene coding a protein comprising the amino acid sequence shown in SEQ. ID NO.:1 (as described in the present application) in the subject, because the presence of the homozygous insertion mutation (the 3-base insertion at positions 805 to 807 in the nucleic acid sequence coding for Angiopoietin-1) is associated with rheumatoid arthritis, as described in the present application and in the experiment described herein.

7. I hereby further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both

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(18 U.S.C. 1001), and that such willful false statements may jeopardize the validity of the above-identified Application or any patent issued thereon.

Date: 27 August 2007

  
Shunichi Shiozawa